Jimma University

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Department of Biology

PHYTOCHEMICAL SCREENIG AND ANTIBACTERIAL ACTEVIY OFROOT EXSTRACT OF Clpurnia aurea in Mizan Town

By:

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Phytochemical Screening and antibacterial activities of root extract of Calpurnia aurea in Mizan Town,Mizan Aman Woreda,Bench Sheko Zone, SNNPR, and Ethiopia.

By:

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Thesis presented to school of Graduate Studies, Jimma University, in partial fulfillment of the requirements for the Degree of Master of Science in General Biology.

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Title of the Thesis

__ PHYTOCHEMICAL SCREENIG AND ANTIBACTERIAL ACTEVIY OFROOT EXSTRACT OF Clpurnia aurea in Mizan Town

Degree Awarded: Msc/PhD (Encircle one)

College of Natural Science, Jimma Unive

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Abstract

The term medicinal plants include a various types of plants used in herbal medicine and some of these plants have medicinal activities. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. The aim of this study was phytochemical Screening of secondary metabolites and to evaluate antibacterial activities of *Calpurnia aurea* root extracts. The extraction of the test medicinal plant for crude extract was carried out by maceration method (a required amount of sample was dissolved in agiven extraction solvent for 72hrs then with 3 or 4hr gap the sample were agitated or shaked to increase the amount of the desired sample). Phytochemical screening for the petroleum ether, ethanol, methanol and distilled water crude extracts of the plant roots were carried following standard methods. Antibacterial activities of the crude extracts were evaluated using the agar disk diffusion method. Foodborne bacterial pathogens used for this study viz. *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Salmonella typhi*. The results of this study showed that petroleum, ethanol, methanol and distilled water extracts used against the test strains at a concentrations of 100, 50 and 25mg/ml were showed antibacterial activity, however, none of the crude extracts had antibacterial activity at a concentrations of 12.5mg/ml against all the selected bacterial species. Petroleum ether extract had better activity at concentration of 100,50, and 25mg/ml (18,12 and 6.5 mean inhibition mm) against *S.aureus, B. cereus, E. coli* and *S. typhi* ,and distilled water extract had good antibacterial activity at 100mg/ml toward the selected test organisms but at 25 mg /ml *S. typhi* was not sensitive. All the extracts had no activity against any of the test strains at 12.5 mg/ml which is a minimum inhibitory concentration. It could be concluded that extracts root of *Calpurnea aurea* had some promising activity against human pathogens and further detailed investigation using sensitivity analytical techniques are recommended for better exploitation of the plant products in various applications.

Keywords*:* Antimicrobial activity, *C. aurea*, Medicinal plants, Pathogens, Phytochemicals

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1. Introduction

Traditional plant refers to the health practices, approaches, knowledge and beliefs incorporating plant and mineral based medicine to treat, diagnose and prevent illness or maintain well-being (Selvamohan et al. 2012).World Health Organization (WHO) has suggested that medicinal plants would be the best source to obtain variety of drugs. Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being (Asres et al., 2001).WHO estimates that 80% of the people in developing countries of the world rely on traditional medicine for their primary health care, and about 85% of traditional medicine involves the use of plant extracts. This means that about 3.5 to 4 billion people in the world rely on plants as sources of drugs (Fouuche et al., 2006). The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Medicinal plant treatments are still used for many health problems (Nostro et al., 2000).

Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defiance mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds (Krishnaiah et al., 2007). According to Giday et al .(2007),plant products derived from barks, flowers, roots, leaves, seeds, fruits, twigs, pods and stems are the part of phytomedicines.

Desta (1994) demonstrate that the medicinal value of medicinal plants depends on bioactive phytochemical constituent's action in the human body. As stated above, phytochemicals are bioactive chemicals of plant origin and they are regarded as secondary metabolites because the plants that manufacture them may have little need for them.According to Beaumont et al. (1999), Phytochemicals are basically divided in to two groups with primary and secondary metabolites based on the function in plant metabolism. The major constituents are primary metabolites such as carbohydrates, amino acids, proteins and chlorophylls while secondary metabolites consist of alkaloids, steroids, flavonoids, tannins, phenol, and terpenoids. Secondary metabolites are non-nutritive plant chemicals that have protective or disease preventive properties. Therefore, the high medicinal value of phytochemicals justifies their scientific investigation.The plant,stem,leaves,and seeds have been the subject of differenet studies in the past. In line with this, the current research is designed to undertake for phytochemical screening and antibacterial activities of different solvent extract of *Calpurnia aurea* root.

1.1. Statement of the problem

Calpurnia aurea plant has been reported to have diverse medicinal uses in traditional medicine (Kubo et al., 1984) and found the focus of this study in order to get primary data to justify the traditional claims. Infectious diseases are disorders that are caused by microorganisms, like bacteria, from one person to another. Humans can also become infected following exposure to an infected animal that harbors a pathogenic organism that is capable of infecting humans. Infectious diseases are a leading cause of death worldwide, particularly in low income countries, especially in young children (Jones et al., 2008). *C. aurea* is one of the most important medicinal plants and to my knowledge there is no recent research report on extraction and antibacterial report on *C.aurea* root. Therefore,this studies was focused to fill the existing reseach gap.

1.2. Objective of the Study

1.2.1. General Objective

The general objective of the study was to determine the phytochemical composition of *Calpurnia aurea* root extracts and evaluate their antibacterial activities against selected pathogens

1.2.2. Specific Objectives

- ✓ To assess the traditional preparation techniqe and use of *Calpurnia aurea as* medicinal plant.
- ✓ To determine the phytochemical components of *Calpurnia aurea* root extracts.
- ✓ To evaluate antibacterial activities of the crude extracts of *Calpurnia aurea* root against food borne bacterial pathogene
- ✓ To determine the minimum inhibitory concentration of crude extracts

1.3. Significance of the Study

Due to development of bacterial resistance to presently available antibiotics has achieved the research for new antibacterial agents or a combination of drugs to be able to combat new resistant pathogenic bacteria. It has been reported in litrature a synergistic effect of various plants extracts with antibiotic and non-antibiotic drugs against some resistant bacteria, therefore was checked this possibility in my study by using traditional medicinal plants.

There are many people's that are still inaccessible for commercial drugs. Therefore; this study was evaluated and recommend an alternative drug from locally available plants at least to minimize the challenges of pathogens.

2. Literature review

2.1. Medicinal Plant

The term medicinal plants include a various types of plants used in herbalism and some of these plants have medicinal activities. Medicinal plants are the “backbone” of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis (Davidson, 2000),these medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world. The Indian sub-continent has a very rich diversity of plant species in a wide range of ecosystems. There are about17, 000 species of higher plants, of which approximately 8,000 species are considered medicinal and used by village communities, particularly tribal communities, or in traditional medicinal systems. The use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed by (UNESCO, 1996).

The World Health Organization observes that it is difficult to assign one definition to the broad range of characteristics and elements of traditional medicine, but that a working definition is essential. It thus concludes that the traditional medicines diverse health practices, approaches, knowledge and beliefs incorporating plant, animal and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness(WHO, 2002). During the past decade, traditional systems of medicine have become a topic of global importance. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Medicinal plants frequently used as raw materials for extraction of active ingredients which used in the synthesis of different drugs. Like in case of laxatives, blood thinners, antibiotics and anti-malarial medications, contain ingredients from plants. As defined by WHO, health is a state of complete physical, mental, and social wellbeing and not merely the absence of disease or infirmity. Medicinal plants can make an important contribution to the WHO goal to ensure, by the year 2000, that all peoples, worldwide, will lead a sustainable socio economic productive life (Lucy, UNSCO). Traditional use of herbal

medicines implies substantial historical use, and this is certainly true for many products that are available as “traditional herbal medicines”. In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs. Although modern medicine may exist side-by-side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons (Vishwakarma et al., 2013).

2.1.1. History and Application of Medicinal Plant

Traditional medicine has remained as the most affordable and easily accessible source of treatment in the primary health care system of resource poor communities. The local people have a long history of traditional plant usage for medicinal purposes. The medicinal use of plants is very old. The writings indicate that therapeutic use of plants is as old as 4000 - 5000 B.C. and Chinese used first the natural herbal preparations as medicines. In India, however, earliest references of use of plants as medicine appear in Rig-Veda, which is said to be written between 1600 - 3500 B.C. Later the properties and therapeutic uses of medicinal plants were studied in detail and recorded empirically by the ancient physicians (an indigenous system of medicine) which are a basic foundation of ancient medical science in India (Prakash and Gupta, 2005).For thousands of years, medicinal plants have been used in various cultures of the world as a safe therapeutic modality. The operation of medicinal plants is based on the rich experiences of innumerable healers over centuries, inherited from ancestors,-to-healer transfer, or developed through personal experiences over time. Modernity or cultural revolutions have not altered the in-depth wisdom of this natural medical paradigm. Consequently, no modern system of medicine can ordinarily lay claim to it. The traditional system of treatment, differing in concept and protocol, exemplifies well-developed systems such as allopathic, homeopathic, ayurvedic, and Chinese systems of treatment (Schippmann et al., 2002; Gurib, 2006). During the past decade, traditional systems of medicine have become a topic of global importance. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Medicinal plants frequently used as raw materials for extraction of active ingredients which used in the synthesis of different drugs. Like in case of laxatives, blood thinners, antibiotics and anti-malarial medications, contain ingredients from plants. Medicine, in several developing countries, using local traditions and

beliefs, is still the mainstay of health care. As defined by WHO, health is a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity. Medicinal plants can make an important contribution to the WHO goal to ensure, by the year 2000, that all peoples, worldwide, will lead a sustainable socio economic productive life (Krishnaaiah et al., 2012).Medicinal plant is an important element of indigenous medical systems in all over the world. The ethno botany provides a rich resource for natural drug research and development (Farnsworth, 1990). Patients use traditional medicine for many reasons. They may be from a remote area where modern medicine is not available when they need it. They may belong to communities whose habits and treatment-seeking behavior resorts to traditional medicine as the first choice. They may prefer traditional medicine believing, for example, that they produce fewer side effects or cures them more effectively. They may have experienced a failure with a modern treatment and want to try traditional methods. They may want to avoid modern health facilities because they perceive them as expensive, unfriendly, dangerous, or ridden with corruption. Patients may also avoid modern drugs sold on the market because they are aware of the fact that many of them are counterfeit or‘‘fake’’ drugs (Graz et al., 2011).

2.1.2. Distribution of Medicinal Plant

The practice of traditional medicine is widespread in China, India, Japan, Pakistan, Sri Lanka and Thailand. In China about 40% of the total medicinal consumption is attributed to traditional tribal medicines. In Thailand, herbal medicines make use of legumes encountered in the Caesalpiniaceous, the Fabaceae, and the Mimosaceae. In the mid-90s, it is estimated that receipts of more than US$2.5 billion have resulted from the sales of herbal medicines. And, in Japan, Herbal medicinal preparations are more in demand than mainstream pharmaceutical products. Africa is a rich source of medicinal plants. Perhaps, the best known species is Phytolacca dodecandra. Extracts of the plant, commonly known as endod, are used as an effective molluscicide to control schistosomiasis (Lemma, 1991). In Africa up to 80% of the population uses traditional medicine to help meet their health care needs (WHO, 2000).Traditional medicinal practices are common in Ethiopia in which about 80% of the population in the country use plant based traditional medicine as their major primary health care system (Toledo et al., 2003). Traditional medicine has been practiced in Ethiopia since long time ago. The knowledge, largely oral, has been transferred from one generation to the next through professional healers, knowledgeable elders and ordinary people. It is estimated that about 80% of the Ethiopian population is still dependent on traditional medicine, which

essentially involves the use of plants (Abebe and Hagos, 1991). According to Dawit (1986), 95% of traditional medicinal preparations are of plant origin. Ethiopia is also a country with many languages, beliefs and highly diversified culture. This diversification contributes to the people of the different localities of the country to develop their own specific knowledge of plant resource uses, management and conservation Ethiopia has a long history of using traditional medicines from plants and has developed ways to combat diseases through it (Asfaw et al., 1999).

2.2. Genus of Calpurnia

Calpurnia is a small genus of shrubs and trees from the family of Fabaceae, which is the family of flowering trees like Cassia, Wisteria, Robnia and Mimosa. Calpurnia is known for its prolific flowering habit and ornamental foliage. Among popular species of this genus, the most commonly found is Calpurnia aurea *(*Palmer and pitman, 1972). The genus of Calpurnia comprises some seven species which are widely distributed in SoutAfrica (Polhill et al., 1981).According to Nana et al. (2010) the plants are mainly trees, shrubs or climbers and grouped into the three sub-families based on morphological characteristics using the appearance of their flowers. Calpurnia fruits are winged and have yellow flowers, pods or leaves.

2.2.1. Calpurnia aurea

Calpurnia aurea is a genus of Flowering Plants within the family of fabaceae. The genus comprises shrubs or small trees in or along the margin of forests in many parts of Ethiopia and widely distributed in Africa from Cape Province to Eritrea and which also occurs in Southern India (Korir et al., 2014). Literature survey brings to light that, all parts of the plant species has been used for different human and animal disease (Gemechu et al., 2013). In native countries like Ethiopia, traditionally, the leave and powdered roots of Calpurnia aurea is used for the treatment of syphilis, malaria, rabies, diabetes, lung TB, hypertension, diarrhoea, leishmaniasis, trachoma, elephantiasis, fungal diseases, different swellings, stomach-ache, abscesses, bowel, bladder disorders, to destroy maggots, to destroy lice, to relieve itches, used as a fish-poison or as a cure for dysentery, exhibit activity against amoebiasis and giardiasis, cough and snake bite (Tadeg, 2004). Calpurnia aurea is commonly known as digitta in Amharic, is an African medicinal plant for over 2500 years *(*Monks et al., 1991; Louis et al., 2007).Calpurnia aurea is a shrub to slender tree of up to 15m tall, widespread along the east coast of Africa, throughout which range it is used in traditional medicine and for various utilitarian purposes. Pplhill et al. (1994) demonstrate that *Calpurnia*

aurea leaves are compounded, up to 20cm long, each having 5-15 pairs of leaflets and a terminal one. The flowers are bright yellow; each about 2.5 cm long, in showy or bright hanging bunches of 8 to 30 flowers. The fruit is a thin pod drying light brown with a papery texture, 5-12 cm long and 0.8-1.9 cm wide, narrowly winged on one side containing up to 8 brownish seeds.

2.2.1.1. Medicinal Uses of Calpurnia aurea

There are several reports on the medicinal use of C. aurea, to kill animal lice, to destroy maggots (larva of housefly) and to treat allergic rashes particularly those caused by caterpillars (Barnes, 1997). In SNNPR, traditionally the root of C. aurea is used for treatment of rabies.It is used by the Shinasha people of Northern Ethiopia to treat amoebiasis and giardiasis while the Amhara people from the same region use the leaves to treat malaria and the seeds to treat hypertension while a combination of the leaves and seeds are used to treat diarrhea, rabies and diabetes (Goosen,1963;Bezuidenhout et al.,1988 and Wen and Walle,2006).The plant has also been used as an insecticide to kill lice, to induce uterine contractions and to treat coughs, amoebic dysentery, syphilis, leishmaniasis, tapeworm, trachoma, ringworm, scabies, elephantiasis, abscesses and wounds as well as stomach ache, vomiting, headache and eye diseases (Hayashi et al.,1974 ;Harper et al.,1976). Its widespread application for diverse ethno medicinal uses has made C. aurea a subject for many pharmacological and phytochemical studies (Walleet al.2007).Calpurnia aurea leaves and powdered roots are used to destroy lice and to relieve itches in South Africa (Jurd et al., 1972). The traditional use of C.aurea seed to treat hypertension in some parts of Shinasha, Agew-awi(Tadeg et al. 2005) and Amhara peoples in northwest Ethiopia.

2.2.1.2. Anti-Bacterial Activities of Calpurnia aurea

According to Adedapo et al. (2008) antibacterial activity of the methanol extracts of the leaves of the C. aurea is much higher than that of the stem. The leaf extract also has activity against the organisms of Serratia marcescens, Pseudomonas aeruginosa and Klebsiella pneumonia at MIC of 5 mg/ml while that of the stem was only active against Bacillus cereus and Streptococcus pyrogens at similar concentrations. Matha (2000) stated that pharmacological studies of C. aurea have shown that the methanol extracts of the leaves and stems have good antibacterial and antioxidant properties.The flavonoids from C. aurea have remarkable biological activities, including inhibitory effects on enzymes, modulatory effect on some cell types, protection against allergies, antiviral, anti-malarial, antioxidant, anti-inflammatory and anti-carcinogenic properties (De Nysschenet al., 1998).Erick (2012) state

that for many years infectious disease have been treated traditionally with plants. Traditionally, species of C. aurea have been used as a remedy for common ailments.

It is, therefore, worth investigating which of the bioactive compounds are responsible for the observed bioactivity. Ethno-pharmacological studies to identify antiviral agents from plant material are extensively carried out. It has also been established that compounds with varied structures show similar activities (Al-Ani et al., 1984). Albuquerque et al. (1981) also state that lupine alkaloids have good biological activity. The main pharmacologically active compounds may be the alkaloid calpurmenine and its 13α-(2'-pyrrolecarboxylic acid) ester though phenolics compounds may also contribute for its pharmacological effects, mainly of antibacterial and antioxidant activities. The alkaloids virgiline and lupanine as well as their carboxylic esters have also been implicated for its effect like as insect attractant/repellent in addition to the above activities (Albuquerque et al., 1981; Elbein et al., 1999).

2.2.1.3. Biological activities of Phytochemicals

Activities of the phytochemicals present in plants in preventing disease and promoting health have been studied extensively to establish their efficacy and to understand the underlying mechanism of their action. (Asres et al., 1986B; Asres et al., 1986A).The bioactive extract should be standardized on the basis of phytochemical compounds. Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate an urgent step for screening of plants for secondary metabolites. The present communication attempts to assess the status of phytochemical properties of medicinal plants to improve the health status of people and also to use in pharmaceutical products of commercial importance in the world (Natesan et al., 2015). Phytochemicals may reduce the risk of coronary heart disease by preventing the oxidation of low density lipoprotein (LDL) cholesterol, reducing the synthesis or absorption of cholesterol, normalizing blood pressure and clotting, and improving arterial elasticity (Radema et al., 1979). Phytochemicals may detoxify substances that cause cancer. They appear to neutralize free radicals, inhibit enzymes that activate carcinogens, and activate enzymes that detoxify carcinogens (Pouche et al., 2006).Promoted for the prevention and treatment of diabetes, high blood pressure, and macular degeneration (Siddiqui et al., 2009).

3. Materials and Methods

3.1. Description of the study site

Bench Sheko zone is one of the major zones in the Southern Nations, Nationalities and People Regions and having its total population of 652, 531 with an area of 19,252 square kilometers (CSA, 2007) and it has 10 Districts and lies between 5.33-7.21 latitude and 34.88 to 36.14 longitudes with an altitude ranging from 500 to 2500 meters above sea level. This zone has seven ethnic groups are existed, such as Bench (45.11%), Ment (21.36%), Amhara(8.23%) ,Kafficho(6.55%), Dizi(5.17%), Sheko(4.21%), Surinam(3.38%) and others(-5.99%). Bench Sheko bordered on the west by South Sudan, on the northwest by Gambella regions, on the north by Sheka, on the north east by Keffa and on the east by Debub Omo. The annual mean temperature ranges between 15.1-27 ^{0}C and the annuals mean rain fall ranges 400-2000mm. Mizan –Aman city administration is located at about 565km from Addis Ababa Regarding the agro-Ecology of the zone, out of the total land size 28.042% is lowland, 15.44% semi humid and 56.74% highland. The main food crops in this Zone include maize, godere (Taro root), and enset, while sorghum, teff, wheat and barley are cultivated to a significant extent. Although cattle, goats and poultry are produced in limited numbers, meat and milk are very much appreciated. Cash crops include coffee, fruits (bananas, pineapples, oranges) and spices (coriander and ginger); honey is also an important local source of income.Bench Maji vegetation area divided in to:dense vegetation/forest/,veg etation, sparse vegetation, Bare soil, and Brunt areas. The area of Bench Maji one of the most original and well-preserved forest areas in the country and these makes the area preferable for ecotourism development.

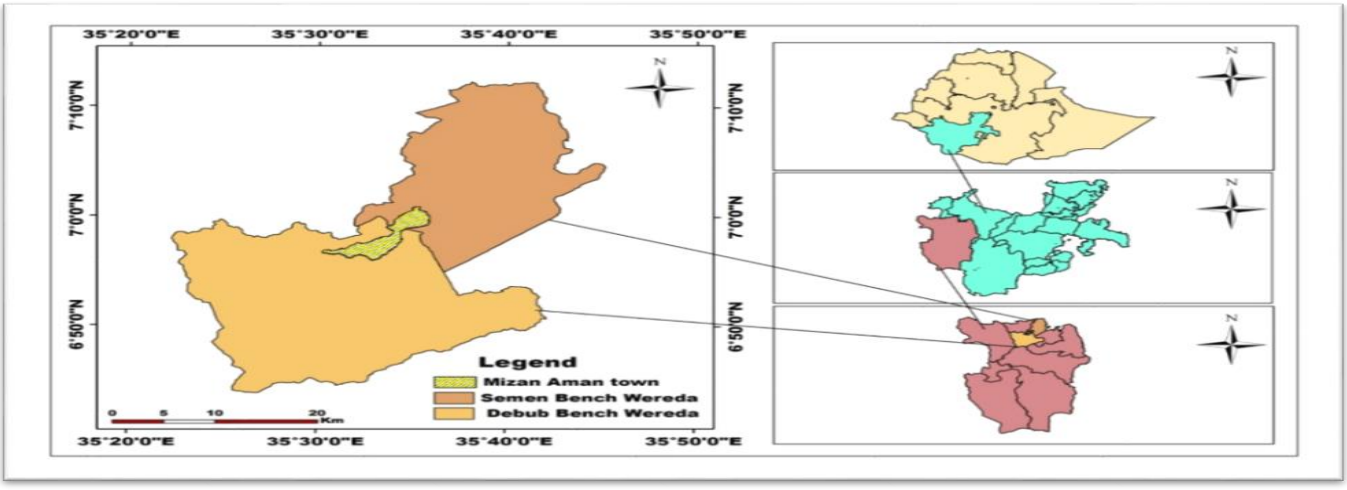

Figure 1**: Map of the study site mizan**

3.2. Study design

A community based cross-sectional study design method was used (semi structure questionnaires was used).This study was used to prepare questionaries' ask traditional healers to collect some information about *Calpurnia aurea* as a medicinal plant used to treat different disease in mizan aman town.and practical work in the laboratory (especially phytochemical screening test and antibacterial activity evaluation).

3.3. Sampling Technique

A purposive sampling method was used for collect information about traditional preparation technique of root of Calpuria aurea to treat different aliments from traditional medicinal healers in the study area during the study period.

3.4. Plant Sample Collection and Preparation

The specimens which are used as medicinal was collected from wild and cultivated areas like home gardens and scientific names was identified by prof. ketesa undera in Jima university. The root of *Calpurnia aurea* was collected and washed with tap water to remove dirt and soil. The collected plant materials were cut into smaller pieces and allowed to dry at room temperature under dark condition to prevent loss of volatile phytochemicals that might be the target constituents. Once dried, the plant material was grinded to ease the extraction processes extracts

3.5. Chemicals and Equipment

Chemicals were used for extractions were petroleum ether, Ethanol, Methanol and distilled water. and chemicals were used in phytochemical screening were, 2%HCl, distilled water, 5%ferric chloride, potassium iodide, iodine, chloroform, sulfuric acid, 1% Lead acetate, sodium hydroxide solution.

Materials and equipment's were used include Electrical grinder, balance, measuring cylinder, flask (1000,500,250 ml,) What man filter paper 1, rota evaporator, beaker , nutrient broth, stove, Bunsen burner, Muller Hinton agar, swamp, forceps, micropipette, chloramphenicol, incubator and ruler, Petri dish, capillary tube, nutrient agar, antibiotic discs.

3.6. Preparation of Crude Extracts

The dry plant material was grinded to a uniform size using an electric grinder. The total sample was (1 kg) powdered *C.aurea* from the total sample was taken 50gm of powdered *C.aurea* root was soaked with the required solvent (petroleum ether, ethanol, methanol and distilled water) (the solvent ratio is 1g:10mL), then 50gm of the root of the plant is soaked 500mL of the extraction solvent, then with occasional shaking at room temperature. After 72hr, the respective crude extracts were filtered by using what man filter paper No.1 and the liquid part was concentrated under Rota vapor to remove the extraction solvent. The resulting crude extracts were weighed transferred to appropriately label vials until further analysis.

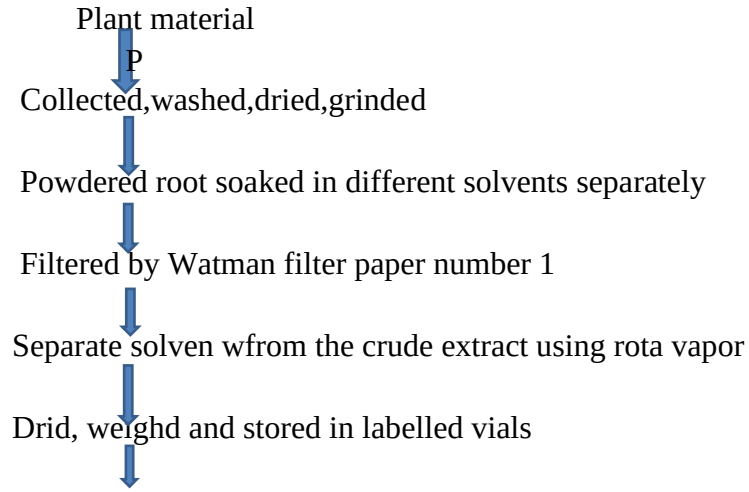

Fig 2: the study frame work for antimicrobial evaluation of plant extracts

3.7. Phytochemical Screening

The crude extracts were screened for the presence of secondary metabolites, such as alkaloid, flavonoids, phenol, steroid, terpenoid, tannin and saponins. The whole procedures were following for phytochemical test.

3.7.1. Phytochemical Test

Alkaloid test: five mL of 2% HCl was added to 2 mL of the extract in a test tube placed on a steam bath and warmed. It was filtered and tests (Jeong et al., 2009).

A few drops of Wagner's Reagent (potassium- iodine solution) were added to one part of the filtrate in a test tube. A reddish brown precipitate was observed indicate the presence of alkaloid.

Flavonoids: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colour less on addition of dilute acid, indicates the presence of flavonoids (Siddiqui et al., 2009).

Phenol test: About 2mL of the extract, 5% ferric chloride solution was added. Deep blue black color indicates the presence of phenol (Steenkamp et al., 2007).

Terpenoid test: Treating the extract in chloroform with few drops of concentrated sulphuric acid, shake well and allow to stand for some time, formation of yellow colored lower layer indicate the presence of terpenoids (Steenkamp et al., 2007).

Steroid test: One ml of the extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow color with green fluorescence indicating the presence of steroids (Nwokocha et al., 2011).

Tannin test: Two ml of extract was added to few drops of 1% lead acetate. A yellowish precipitate indicated the presence of tannins (Nicholas et al., 2013).

Saponins: Dilute the extract with distilled water and shake in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins (Steenkamp et al., 2007).

3.8. Antibacterial Assay

The disc diffusion technique has been widely used to assay plant extract for antimicrobial activity (taiwo et *al*., 2007)

3.8.1. Agar Disc Diffusion

Diffusion discs of 6 mm diameter were prepared from absorbent filter paper (Whatman no.1) by using a paper Puncher and sterilized at 120 °C for 1 h and dried in an oven. Then after, sterilized discs were soaked aseptically by applying 30 µl of each crude extract of plant at a concentration of 100 mg/ml using sterile digital micropipette and then allowed to dry at a room temperature for 15 minutes and then placed in sterile container and stored at 4 ^{o}C until further use (CLSI, 2012).

Chloramphenicol (30µg/mL) was used as positive control for all bacterial species and 10% aqueous DMSO was used as negative control The two gram-positive strains were *Bacillus cereas,* and *Staphylococcus aureus*. The two Gram- negative strains were *Escherichia coli,* and *Salmonella typhi*. All test strains were transferred separately into sterile nutrient broth and incubated at 37°C for 24h. Four were-isolated distinct colonies of the same morphological type were picked from an agar plate culture. The picked colonies were transferred to another sterile nutrient broth and incubated for few hours until the broth turns turbid, and then the inoculum was adjusted to 0.5 Mac Farland (produce from .05 ml of Barium chloride ($Bacl_2$) and 99.5ml of dilute sulfuric acid or 1% H_2so_4) and compare bacteria with 0.5 Mac farland before swabbing another Muller hinton agar plate.

The antimicrobial activities of the four crude extracts (petroleum ether, methanol, distilled water and ethanol) test samples from *C. aurea* root were carried out by disc diffusion method: Muller Hinton agar were prepared according to the manufacturers recommendation by dissolving the required amount of the powder in distilled water was boiled to mix thoroughly and then, Muller Hinton Agar media, petri dish, forceps, paper discs(6mm diameter)were sterilized by autoclave at 121°C for 1h (Otang et al., 2011).

The sterilized Muller Hinton Agar media (25ml) was poured on to the sterilized petri plates under a laminar hood and allowed to solidify at room temperature for 30 minutes. About 100µl of an overnight activated test organisms, namely *Staphylococcus aureus* ATCC 5923, *Bacillus cereus* ATCC14579, *Escherichia coli* ATCC 5922 and *Salmonella typhi* ATCC 3311 were transferred into the pre-sterilized MHA media and were spread gently across the entire plate. Each of the extracts prepared at four different concentrations (100, 50, 25 and 12.5mg/ml) was transferred in duplicate on to sterile paper disc (6mm) and were placed on the surface of the inoculated MHA media using sterile forceps, and gently pressed down onto the agar surface to make sure that the disk will not move. Disks soaked with 10%DMSO were used as a negative control and Chloramphenicol (30 µg/mL) as a positive control. The plates were incubated at 37 ^{0}C for 24 hours and clear inhibition zones around the paper disc were measured after 24hr. Then after, inhibition zones diameters of the extracts were measured (mm) using ruler the values recorded to the nearest whole millimeter for each extracts, the positive control drugs and the negative control (DMSO) as suggested by Steenkamp et al.(2007). Each treatment consisted of three replicates and repeated at least twice.

3.8.2. Determination of Minimum Inhibitory Concentrations (MIC's)

MIC is defined as the lowest concentration of the antimicrobial agent that inhibits the microbial growth after 24 h of incubation. The most effective plant extracts which exhibited a strong antibacterial activity at 100 mg/ml was further evaluated to determine their MIC using disk_diffusion of 6 mm diameter were prepared from absorbent filter paper (Whatman no.1) by using a paper Puncher and sterilized at 120 ℃ for 1 h and dried in an oven and apply each extract and measuring the inhibition zone of the extracts on a plates the lowest concentration of *C. aurea* plant root extracts inhibiting the growth of the organism in asolid media was determined. The lowest concentration of the extract for which growth was observed was considered as MIC of the extract.

3.9. Methods of Data Analysis

Analyses were performed by using descriptive statically methods and data were presented in table and pictures.

4. Results

4.1. Traditional preparation and uses of *Calpurnia aurea* root

Traditional knowledge of medicinal plants and their use by traditional healers use to treat populations for their primary health cares.

Figure 3: photographs showing *C. aurea* tree [picture taken by Yohannis Afework, 15 04 2019]

- With regards to the procedure of application of *C. aurea,* the traditional healers collect *C. aurea* root, wash with tap water and crush it to fine powder. The powder is mixed with water and used to treat infected people, mainly for the treatment of rabies.

Table 1: Information gathered from the traditional healers, the dose given by traditional healers have there own measurement to treat an infected people depends on the patient's age but for easy to understand I was converted in to ml.

Patient age(year)	Dose of traditional medicine in ml
4-6	20
7-15	35
Above 16	50

- The treatment continuous for a while depending on the level of improvement revealed by the infected person. Under serious cases, especially if the patients are vomiting, the provision of treatment carried out for seven days (based on information gathered from Traditional healer in the study site).

4.2. Extraction of plant Matter

The powdered roots of *Calpurnia aurea* root (50 gm.) it is possible another amount but I was select this amount were soaked separately with different extraction solvents (petroleum ether, ethanol, methanol and distilled water) to obtain the respective yields of crude extracts through maceration after concentrated at rota vapor extraction techniques (Table 2). Although all the solvents used for extraction purpose yielded low yields (2-4%), relatively the highest yield (4%) was recorded for distilled water and the least (2%) for petroleum ether.

Table 2: The % yield of *C. aurea* roots extracted with different solvents

amount of root (gm)	Solvent	Weight of dried crude extract(gm)	% yield	Extraction method
Root (50)	Distilled Water	2	4	Maceration
Root (50)	Petroleum ether	1	2	Maceration
Root (50)	Methanol	1.8	3.6	Maceration
Root (50)	Ethanol	1.7	3.4	Maceration

The crude extracts (Table2) were used for phytochemical screening (qualitative identification of phytochemical constituents present in each extracts) and antibacterial activity evaluation to determine whether the crude extracts had bioactive compounds active against selected bacterial test strains.

4.3. Phytochemical test

The extracts of root of *C. aurea* were assessed for the presence of various secondary metabolites.

Table 3: Phytochemical component of root extract of C. aurea

Phytoconstituent Evaluated (tested)	Solvent used for extraction, characteristics of the crude extracts and relative proportion (intensity)			
	Petroleum ether	Ethanol	Methanol	Water
	Intensity	Intensity	Intensity	Intensity
Alkaloid	+++	++	+++	+++
Flavonoid	+++	+++	+++	+++
Phenol	-	++	-	-
Terpenoid	-	-	-	+
Steroid	-	+++	-	+
Tannin	+	+++	++	++ +++
Saponin	+	+	++	

Key: +++ highly present ; ++ present in medium amount; + present in low amount, - absent

The phytochemical screening gives a qualitative estimation of phytochemical constituents present in the plant extract. Accordingly, Petroleum ether extracts of root of *Calpurnia aurea* were found very rich in alkaloids and flavonoid, But, tannin and saponins were present in low proportions while steroid, terpenoid and phenols were absent in the same extract (Table 3). The constituents that were present in higher proportions in the ethanol extracts were flavonoid, tannin, steroids, and phenol and alkaloids were present in medium proportions, and saponin was present in low amount while terpenoids were absent (Table 3). Likewise, methanol extracts of root of the same plant showed the presence of alkaloids and flavonoids in higher proportions while saponin and tannins were present in medium amount but steroids, phenol and terpenoids were missing (Table 3). Furthermore, alkaloids, saponins and flavonoids were detected in distilled water extract of the plant in higher proportions and tannin was present in medium amount while terpenoids and steroids were present in lower proportions and phenolic compounds were absent (Table3).

4.4. Antibacterial Activities of Root Extracts of *Calpurnia aurea*

It was clear from the present results that all the root extracts of *C. aurea* exhibited pronounced activity against the gram positive and gram-negative bacterial species.

Table 4: Antibacterial activities of root extract of C. aurea using different solvent

Solvent used	Extract Conc (mg/ml)	Inhibition Zone diameter (mm) against test strains				
		S. aureus	B.cerus	E.coli	S. typhi	Mean(mm)
Petroleum ether	100	14	19	21	18	18
	50	10	13	14	11	12
	25	6	7	8	5	6.5
	12.5	NA	NA	NA	NA	0
	+ve control	26	11	26	23	21.5
	_ve control	0	0	0	0	0
Ethanol	100	11	18	21	17	16.5
	50	8	10	13	12	10.75
	25	4	5	6	6	5.25
	12.5	NA	NA	NA	NA	0
	+ve control	26	11	26	23	21.5
	-ve Control	0	0	0	0	0
Methanol	100	15	17	20	18	17.5

	50	8	10	14	13	11.25
	25	4	4	7	6	5.25
	12.5	NA	NA	NA	NA	0
	+ve control	26	11	26	23	21.5
	-ve Control	0	0	0	0	0
Distilled water	100	16	12	18	13	14.75
	50	13	8	14	9	11
	25	6	5	9	NA	5
	12.5	NA	NA	NA	NA	0
	+ve control	26	11	26	23	21.5
	-ve Control	0	0	0	0	0

4.4.1. Antibacterial activities of petroleum ether, ethanol, methanol and distilled water extract against the test strain

As briefly summarized above, *E.coli* was more sensitive to the petroleum ether extract at 100mg/ml concentration as compared to another bacterial species with the inhibition zone diameters (mm) recorded for the four bacterial species being 14, 19, 21 and 18 for *S.aurse, B. cerus , E.coli* and *S.typhi*, respectively. As concentration decreases from 100mg/ml to 50mg/ml the activities of the extract also decreased down with inhibition zone diameters (mm) of 10, 13, 14 and 11 against *S. aureus, B. cereus , E.coli* and *S. typhi*, respectively. As the concentrations of the root extract decreases the phytoconstituents that inhibit bacterial growth also decrease, hence their activity.

The activities of the extract significantly decreased as the concentration of the extract further diluted down to 25mg/ml with inhibition zone diameter (mm) of 6,7,8 and 5 against *S.aurse ,B. cerus, E.coli and S.typhi*, respectively, with almost no activity recorded for extract's concentration of 12.5mg/ml(Table 4). As briefly summarized from the above table, *E.coli* was more sensitive to the ethanol extract at 100mg/ml concentration as compared to another bacterial species with the inhibition zone diameters (mm) recorded for the four bacterial species being 11, 18, 21 and 17 for *S.aurse, B. cerus , E.coli* and *S.typhi*, respectively. As concentration decreases from 100mg/ml to 50mg/ml the activities of the extract also decreased down with inhibition zone diameters (mm) of 8,10,13 and 12 against *S. aureus, B. cereus , E.coli* and *S. typhi*, respectively. As the concentrations of the root extract decreases the phytoconstituents that inhibit bacterial growth also decrease, hence their activity. The

activities of the extract significantly decreased as the concentration of the extract further diluted down to 25mg/ml with inhibition zone diameter (mm) of 4,5,6 and 6 against *S.aurse ,B. cerus, E.coli and S.typhi*, respectively, with no activity recorded for extract's concentration of 12.5mg/ml (Table4). As briefly summarized above, *E.coli* was more sensitive to the Mthanol extract at 100mg/ml concentration as compared to another bacterial species with the inhibition zone diameters (mm) recorded for the four bacterial species being 15, 17, 20 and 18 for *S.aurse, B. cerus , E.coli* and *S.typhi*, respectively.

As concentration decreases from 100mg/ml to 50mg/ml the activities of the extract also decreased down with inhibition zone diameters(mm) of 8,10,14 and 13 against *S. aureus, B. cereus , E.coli* and *S. typhi*, respectively. As the concentrations of the root extract decreases the phytoconstituents that inhibit bacterial growth also decrease, hence their activity. The activities of the extract significantly decreased as the concentration of the extract further diluted down to 25mg/ml with inhibition zone diameter (mm) of 4,5,7 and 6 against *S.aurse ,B. cerus, E.coli and S.typhi*, respectively, with no activity recorded for extract's concentration of 12.5mg/ml (Table4). In cause of Distilled of water, *E.coli* was more sensitive to the Distilled water extract at 100mg/ml concentration as compared to another bacterial species with the inhibition zone diameters (mm) recorded for the four bacterial species being 16, 12, 18 and 13 for *S.aurse, B. cerus , E.coli* and *S.typhi*, respectively. As concentration decreases from 100mg/ml to 50mg/ml the activities of the extract also decreased down with inhibition zone diameters(mm) of 13,8,14 and 9 against *S. aureus, B. cereus , E.coli* and *S. typhi*, respectively. As the concentrations of the root extract decreases the phytoconstituents that inhibit bacterial growth also decrease, hence their activity. The activities of the extract significantly decreased as the concentration of the extract further diluted down to 25mg/ml with inhibition zone diameter (mm) of 6, 5, 9 and NA against *S.aurse ,B. cerus, E.coli and S.typhi*, respectively, In general, the bioactivities of Petroleum Ether ,Ethanol, Methanol and Distilled water Extract of root of *Calpurnia aurea* against the four test strains showed good activity at high concentration of the plant extract but the bioactivity analysis distilled water extract was no active at 25mg/ml with *S. typhi* but the other extracts were active at 25mg/ml and no activity recorded for extract's concentration of 12.5mg/ml. (Table4).

Discussion

Secondary metabolites of medicinal plants contribute significantly for biological activities of the plants including: hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic, anti-malarial, anti-cholinergic, anti-leprosy activities (Negi et al., 2011).Among the phytochemical commonly isolated from medicinal plants, in general, and root of *C.aurea*, in particular, are flavonoids. Flavonoids have been consumed by humans since the advent of human life on earth, about 4 million years. Flavonoids have been reported to exert wide range of biological activities including anti-inflammatory, antibacterial, antiviral, antiallergic (cushine et al., 2005), cytotoxic antitumor, treatment of neurodegenerative diseases, vasodilatory action (Tsuchiya, 2010 and Chebil et al., 2006). In addition, flavonoids are known to inhibit lipid-peroxidation, platelet aggregation, capillary permeability and fragility, cyclo-oxygenase and lipoxygenase enzyme activities. They exert these effects as antioxidants, free radical scavengers, chelators of divalent cation (Chebil et al., 2006 and Middeton et al., 2000).

Saponins were the other phytochemical detected in the root extract of *C.aurea,* contributing to the antimicrobial activities of the extract observed at higher concentrations. Saponins have been ascribed for numbers of pharmacological action (Kensil, 1996; Setezer and Setezer, 2003; and Fuchs et al., 2009). The important ones being permeabilizing of the cell membrane (Hostettman and Marston, 1995), lowering of serum cholesterol, stimulation of luteinizing hormone release leading to obortifacient properties (Francis et al.,2002), immunomodulatory potential via cytokine interplay , cytostatic and cytotoxic effect on malignant tumor cell (Bachran et al.,2008), adjuvant properties for vaccines as immunostimulatory complexes (Sjolander et al., 1998) and synergistic enhancement of the toxicity of immunotoxins (Heisler et al., 2005).

Although not as frequent as other phytochemicals, phenolics were also among the phytochemicals encountered in the root extracts of *C.aurea.* Phenolics have been considered powerful antioxidants in vitro and proved to be more potent antioxidants than Vitamin C and E and carotenoids (Rice-Evans et al., 1995).Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer, and also to have antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, antiinflammatory, and immunomodulatory properties (Rabi and Bishayee,2009; Shah et al., 2009). In addition, terpenoids can be used as protective substances in storing agriculture

products as they are known to have insecticidal properties (Theis and Lerdau, 2003). According to Erick (2012), the presence of these Terpenoids in *Calpurnia* could contribute to the observed antibacterial activity. As the study has revealed flavonoid (highly detected in all solvents), alkaloid (highly detected in petroleum ether, methanol and distilled water), phenol (medium detected in ethanol), steroid and tannin (strongly detected in ethanol), saponin (strongly detected in distilled water) and terpenoid(weakly detected in distilled water). Most tested plant extracts showed antibacterial activity against *S.aureus,B. cereus,E. coli* and *S. typhi* which may reflect the antibacterial activity of plant active ingredients that inhibit bacterial growth. The petroleum crude extract C. aurea root exstract in 100,50,and 25mg/ml were showed the highest activity against S.aureus, B. cereus, E. coli and S. typhi with a mean inhibition zone 18,12,and 6.5 mm respectively (Table:4). and then the negative and positive controls showed a zone of inhibition = 0 and 21.5 mm respectively (Table:4). Ethanol crude extract C. aurea root exstract in 100,50,and 25mg/ml were showed the a good activity against S.aureus, B. cereus, E. coli and S. typhi with a mean inhibition zone 16.5,10.75,and 5.25 mm respectively (Table:4). and then the negative and positive controls showed a zone of inhibition = 0 and 21.5 mm respectively (Table:4). The methanol extracts of C. aurea root (extracted for 72 h) showed the highest effect towards S. aureus B. cereus, E. coli and S. typhi with a mean inhibition zone 17.5,11.25,and 5.25 mm respectively (Table:4). and then the negative and positive controls showed a zone of inhibition = 0 and 21.5 mm respectively (Table:4). The distilled water extracts of C. aurea root (extracted for 72 h) showed less activity compare to other extract towards S. aureus B. cereus, E. coli and S. typhi with a mean inhibition zone 14.75,11, and 5 mm respectively (Table:4). and then the negative and positive controls showed a zone of inhibition = 0 and 21.5 mm respectively (Table:4). In general the root extracts Showed the highest antibacterial activity by petroleum ether methanol, ethanol and distilled water extracts (mean inhibition zone 18, 17.5, 16.5 and 14.75for four bacterial strain). The study revealed that antibacterial activity of the crude extract from the Calpurnia aurea roots were variable when extracted by different solvents, however; possesses good antimicrobial activity which supports the traditional uses of *Calpurnia aurea* in the treatment of rabies and other infections under study.

The result of the present study support the traditional usage of Calpurnia aurea plant which possesses secondary phytochemical with antimicrobial property that can be used as antibacterial agent in new drugs from the therapy of infectious diseases caused by pathogens and further work may be carried out for pharmacological evaluation.

Conclusions

Traditional knowledge of medicinal plants and their use by traditional healers use to treat populations for their primary health cares.As per the information gathered from the traditional healers, Calpurnia aurea root to teat rabies.

In petroleum ether crude extracts of the *C. aurea* roots of the plant have revealed the presence of alkaloids, flavonoid, tannin and saponins but steroid, terpenoid and phenols were abscent. In ethanol extract presence of flavonoid, tannin, steroids, saponin and alkaloids while terpinoids was absent. In methanol crude extracts of the root of *C. aurea* showed the presence of alkaloids, flavonoids, saponins and tannins but no phenol, steroids and terpenoids. Likewise, distilled water exstract showed the presence alkaloids, saponins, flavonoids, terpenoids and steroids while phenolic compound was absent.

E.coli was highly sensitive to petroleum ether extract at a concentration of 100mg/ml (with IZ diameter of 21mm) as compared to another three bacterial species including *S.aureus* (IZD =14mm), *B. cereus* (IZD = 19mm), and S. typhi (IZD =18mm) and standard antibiotic Chloramphenicol (20µg/ml) (IZD=26mm).

The antimicrobial activities decrease with dilution of the initial concentration down to 50 and 25mg/ml and totally lost activity at 12.5mg/ml. The bioactivity of ethanol, methanol and distilled water extracts of the at a concentrations of 100, 50, 25 mg/ml had good activities against the selected bacterial species (*S. aureus, B. cereus, E. coli* and *S. typhi*) at higher concentration, the most activity being observed at 100mg/ml) but no activity at all concentration at 12.5mg/ml concentrations.

Recommendations

The biologically active compounds in *C.aurea* root extract support the medicinal application of the plant. The study confirmed bioactivity of the crude extracts due to the presence of bioactive compounds in the crude extracts of *C. aurea* roots. Previous studies on the stems and the stem bark of the plant also reported the anticancer, antioxidant, analgesic, antibacterial and anti-helminthic properties of extracts from the same plant. The current and previous observations indicate the plant's promising potential as the source of many other divers biologically active compounds of significant application in food and pharmaceutical industries. Cognizant of this, huge potential, further isolation, characterization and structural elucidation of active compounds and clinical studies on the isolated compounds are recommended.

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APPENDIX 1

Check List to Collect Information on Medicinal Plant

JIMMA UNIVERSITY

COLLAGE OF NATURAL SCIENCE

DEPARTMENT OF BIOLOGY

The objective of this study is to collect some information about *Calpurnia aurea* as a medicinal plant used to treat different bacterial infections. Therefore; the researcher kindly requests you to give true and valuable information in response to the questions.

I. DEMOGRAPHIC INFORMATION

1. Name of the respondent----------sex----------

   Kebele----------Occupation----------Age----------

RESEARCH QUESTIONS

The current study will address many research questions with regards to the use of medicinal plants being used for treatment of different bacterial infections.

THE RESEARCH QUESTIONS ARE:

1. How do you traditionally use (preparation methods) the root of *Calpurnia aurea*?
2. What are the local uses of root of *Calpurnia aurea* (other than for the treatment of diseases) ?
3. What are the common diseases protected by the root of *Calpurnia aurea*?

APPENDEX: 2

Image phytochemical screening of secondary metabolites

Fig 2: phytochemical screening of secondary metabolite

APPENDEX: 3

Images of antibacterial activity of roor extract Calpurnia aurea

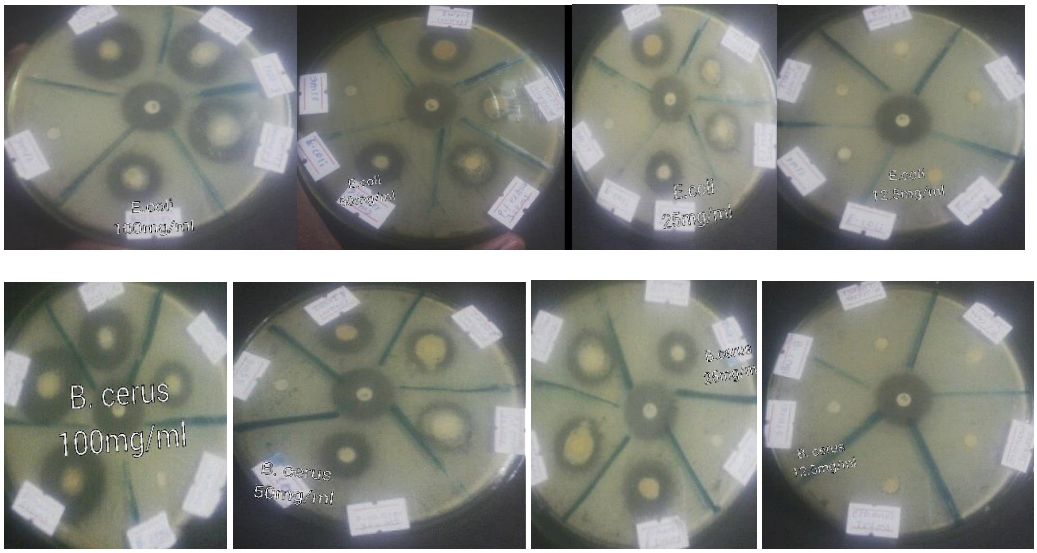

Figure 2:- shows examples of the antibacterial activity of Calpurnia aurea root extract of E. coli and B. cerus at concentration of 100,50, 25,and 12.5 as a sample.